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BIOENERGY/BIOFUELS/BIOCHEMICALS

Evaluating biochemical methane production from brewer's spent yeast

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Abstract Anaerobic digestion treatment of brewer's spent yeast (SY) is a viable option for bioenergy capture. The biochemical methane potential (BMP) assay was performed with three different samples (SY1, SY2, and SY3) and SY1 dilutions (75, 50, and 25 % on a v/v basis). Gompertz-equation parameters denoted slow degradability of SY1 with methane production rates of 14.59–4.63 mL/day and lag phases of 10.72–19.7 days. Performance and kinetic parameters were obtained with the Gompertz equation and the first-order hydrolysis model with SY2 and SY3 diluted 25 % and SY1 50 %. A SY2 25 % gave a 17 % of TCOD conversion to methane as well as shorter lag phase (<1 day). Average estimated hydrolysis constant for SY was 0.0141 (±0.003) day^{-1}, and SY2 25 % was more appropriate for faster methane production. Methane capture and biogas composition were dependent upon the SY source, and co-digestion (or dilution) can be advantageous.

Keywords Anaerobic digestion · Biochemical methane potential (BMP) · Brewer's spent yeast

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Introduction

Since significant amounts of by-products and solid wastes are generated by the brewing industry, their disposal and management represent important cost and operation factors [1] and also an environmental challenge. Brewer's spent yeast (SY) is the second largest by-product from breweries [2], being currently generated at a rate of 1.5–3 % of the total volume of beer produced [1]. Once discarded from the brewing process, SY cannot be treated as a liquid waste, since it is considered a contaminant when mixed with water effluents because of its contribution to the Biochemical Oxygen Demand (BOD) in water bodies [3]. In addition, a rapid change in composition of this waste occurs when no preservation method is used and is kept at room temperature [4]. Thus, an inactivation treatment of the SY is required before disposal or even management. This is commonly energy intensive, as it involves the addition of chemical substances or heating [2, 5, 6].

During the brewing fermentation, yeast catalyzes the conversion of sugars in wort to carbon dioxide and alcohol [7]. The SY is recovered almost at the end of the process, and only a fraction can be reused [8]. SY waste includes yeast solids, beer solids, and sediment of hops and grains as particulate matter. It has a high content of protein, vitamins

and amino acids, as well as carbohydrates, but their composition varies with the physiologic condition and phase in growth cycle of the yeast [9]. Dry matter content of this by-product can reach up to 10 % w/w [1].

After inactivation through pasteurization and drying, SY has been commercially used for animal feed or as a nutritional supplement [2]. When yeast is sold for human food or other specialized biotechnological uses, it requires an intensive processing [2, 5, 10]. The fraction of this waste that is not sold is currently disposed in landfills, and with less frequency, directed to anaerobic digesters in wastewater treatment plants for methane generation [11].

Particularly, when a high volume of SY is produced, other feasible alternatives should be explored, as the costs of transport, storage, and post-processing become significant. The most important reasons to use anaerobic digestion to treat organic solids wastes are: (1) a required environmental friendly disposal; (2) possible recovery of renewable fuel as biogas; (3) relatively low costs in starting up and managing the process; and (4) stabilization and pathogen inactivation of the resulting solids, which has enhanced value for beneficial agricultural and livestock applications [12]. This process involves the transformation of complex and large-sized organic solids into simpler and smaller compounds by a multi-step biological mechanism performed by bacteria and archaea. The final expected products are biogas, and stabilized solids residue rich in nutrients [13]. The biogas produced, that is composed of carbon dioxide, methane, and small amounts of hydrogen sulfide and ammonia as well as water vapor, can be used as a fuel in combined heat and power (CHP) production.

There are only a few publications that report anaerobic digestion treatment of SY to obtain methane, and better results were obtained when it was co-digested [11, 14, 15]. Other reports documented that chemical, thermal, and mechanical pretreatment of the SY did not improve the methane production [11], and long hydraulic retention times were suggested due to slow biodegradability [14].

Biochemical methane potential (BMP) assay has been widely applied as a standard protocol to estimate biodegradability and specifically, to evaluate biogas yields or performance of different substrates in anaerobic digestion [16]. The first objective of this work was to confirm the feasibility of Brewer's SY to undergo anaerobic digestion through BMP assays and to test various dilution ratios of SY should there be an inhibition to methanogenesis. A second goal of this study was to fit the experimental data obtained to the Gompertz equation, a currently used empirical model that facilitates the interpretation and BMP results extrapolation. The third goal of this work was to perform short BMP assays with SY from three different beer sources, since the composition of the SY varies depending on the type of beer source as well as the sample collection method. Remnants of beer additives like hops could affect methane potential, since hops resins inhibit bacterial activity [7, 17]. The impact that this could have on methane production was evaluated applying the Gompertz equation as well as a kinetic model to obtain the hydrolysis rate constant for each SY sample. As far as we know, this work presents the first detailed characterization of different SY samples as well as an interpretation of methane production results using mathematical models. The results obtained could be useful for the brewing industry to find strategies for the management of this waste with a positive environmental impact.

Materials and methods

Spent yeast samples and characterization

Samples of SY from three different beer sources were obtained from Four Peaks Brewing Company in Tempe, AZ., to be characterized and subjected to the BMP test. Each of these SY samples came from different beers which had different flavor additives. The first sample of spent yeast (SY1) came from a beer made with three types of barley malt and had 48 International Bitterness Units (IBU), the second one (SY2) from a beer that was prepared using a combination of white wheat malt and barley malt and had 17 IBU [18], and the third one (SY3) from a beer made with four types of barley malt and that had 55 IBU [18]. Total chemical oxygen demand (TCOD), total nitrogen (TN), ammonia nitrogen (NH3-N), alkalinity ($CaCO_3$), and total volatile fatty acids (VFA) were measured using an HACH kit and spectrophotometer. Total suspended solids (TSS) and volatile suspended solids were measured per standard methods (A P H A [19]). Ethanol and other volatile fatty acids concentration for both samples was quantified using an HPLC (Shimadzu Corporation, MD, USA) equipped with an AMINEX HPX-87H column at 50 °C with 2.5 mM H_2SO_4 at 0.6 mL/min.

A colorimetric method was used to determine carbohydrate concentration [20] by placing 2 mL of sample in a 15 mL culture tube, and adding 50 µL of 80 % phenol solution (w/w), and consecutively 5 mL of 95.5 % sulfuric acid. A standard calibration curve with glucose was developed, and spectrophotometer was used to measure using a wavelength of 485 nm. The bicinchoninic acid method was used for proteins [21], using a BCA protein assay kit (Sigma-Aldrich, St. Louis, MO) for analysis. A standard curve with Bovine Serum Albumin (BSA) was developed with all measurements in a spectrophotometer using a wavelength of 562 nm.

Table 1 Experimental setup for batch BMP tests

Assay ID	Spent yeast (mL)	Water (mL)	Additions (g/L)	Anaerobic basal medium (mL)	Inoculum (mL)
SY1	126	0	0.7 NH_4Cl	0	54
SY1 75 %	94.5	31.5	0.7 NH_4Cl	0	54
SY1 50 %	63	63	0.7 NH_4Cl	0	54
SY1 25 %	31.5	94.5	0.7 NH_4Cl	0	54
SY2 25 %	31.5	94.5	0.7 NH_4Cl	0	54
SY3 25 %	31.5	94.5	0.7 NH_4Cl	0	54
Negative control	0	0	0	126	54
Positive control	0	0	2. 72 acetate	126	54

Biochemical methane potential experimental setup

From a BMP assay conducted in batch conditions at bench scale, it is possible to obtain the amount of methane produced per milligram of Chemical Oxygen Demand (COD), a characteristic of the organic substrate, and compare it with theoretical methane production [12]. Based on BMP batch assay protocol [22, 23], a negative control or blank was prepared feeding only Anaerobic Basal Medium to the anaerobic digested sludge inoculum. The blank methane production is subtracted from the methane obtained from the samples (+ anaerobic inoculum) assays. A positive control was also performed consisting of anaerobic inoculum and anaerobic basal media augmented with acetate (2.72 g/L or 20 mM). The anaerobic sludge inoculum was obtained from Mesa (AZ) Northwest Wastewater Reclamation Plant (MNWWRP) with 35.1 ± 0.6 g of TCOD/L and 18.1 ± 0.3 g of VSS/L.

A summary of the experimental setup for the BMP batch tests is presented in Table 1, and a graphic representation is shown in Fig. 1. For every test, a 126 mL sample and 54 mL inoculum were added to 250 mL serum bottles corresponding to a 2.3:1 substrate to inoculum volume ratio and an inoculum/substrate ratio of 1.03 on a g VSS basis. Previous to the BMP test, a pH adjustment of the samples was performed adding NaOH (10 M) in small drops up to a volume of 0.2 % of the sample volume under constant agitation to reach a neutral pH (7 ± 0.2). The pH was measured with an epoxy body sealed combination electrode (Cole Parmer, Vernon Hills, IL). Each bottle containing sample was added with 0.7 g/L of NH_4Cl, as a nitrogen supplement. N_2/CO_2 (80/20 % volume) gas was purged in the serum bottles before sealing them with rubber stoppers and aluminum caps to create anaerobic conditions and to maintain a neutral pH. The serum bottles were incubated at 37 ± 1 °C and shaken at 180 rpm during the BMP test. The tests were done in duplicate.

Initially, three different dilutions of SY1 were tested only, since it was the first obtained sample and the dilutions would allow us to select an appropriate COD load for the incoming samples. The dilutions were prepared using deionized water (25, 50, and 75 %) in an assay with SY1 that lasted 45 days. Independent 20 days BMP tests were performed with SY1 50 % diluted, and other different samples of SY (SY2 and SY3) each adjusted to approximately 17 ± 1 g TCOD/L (which represents a 25 % dilution) to prevent substrate overloading and based on the results obtained from the SY1 initial test.

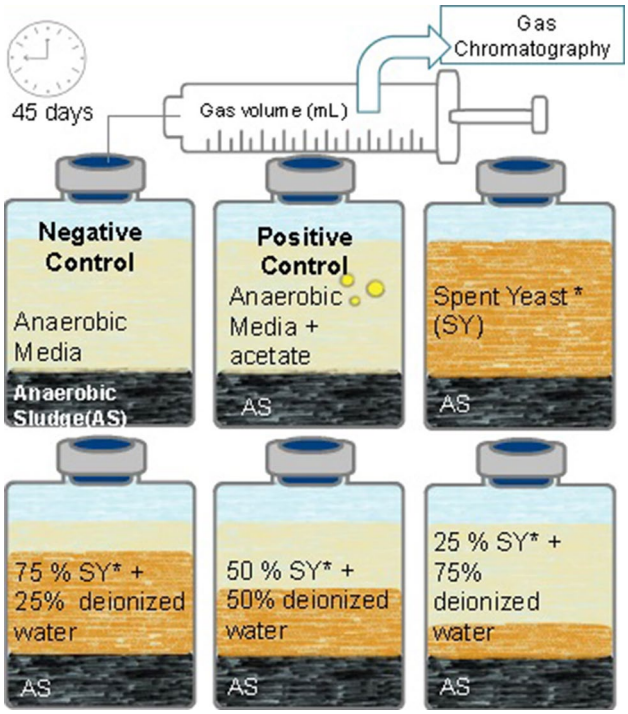

Fig. 1 BMP experimental setup of the 45 days experiments with SY1 and its dilutions (75, 50, and 25 %); the positive and negative controls are also represented

Biogas production

Periodic measurements of the biogas production volume were done using a frictionless glass syringe (Perfektum,

NY), in the headspace of the serum bottles after allowing the syringe to equilibrate with atmospheric pressure. To obtain the CH_4 concentration, the biogas produced was analyzed by gas chromatography (GC-2010, Shimadzu, Japan) equipped with a thermal conductivity detector (TCD) and a packed column (Shincarbon ST 100/120, 2 m, Restek, Bellefonte, PA). During the analysis, the GC-TCD was operated at 145 °C iso-thermal condition (inlet 120 °C; detector 150 °C; current 45 mA), and N_2 was used as the carrier gas. The standard curves were prepared using certified CH_4, CO_2, and H_2 mixed gas (40 %:30 %:30 %, Matheson Tri-Gas, Twinsburg, Ohio).

Mathematical models used for BMP data fit

The batch BMP data obtained can be interpreted by fitting empirical equations that help to understand basic mechanisms [24, 25]. The Gompertz equation (1) is not a predictive model, but it is often used to obtain parameters determining methanogenesis performance [26] and was employed in this study to fit the experiment results that lasted 45 days with SY1 and its respective dilutions, as well as with SY2 and SY3 20 days assays

$$M_P = P_M \cdot \exp\left\{-\exp\left[\frac{R_M \cdot \exp}{P_M}(x_o - x) + 1\right]\right\} \quad (1)$$

where M_P is the observed cumulative methane production (mL), and P_M is the ultimate methane production (mL). R_M is the observed methane production rate (mL/day), and x_o is the lag phase time (days). The x is the time of measurements (days), and exp is the exponential (2.718). These parameters can be evaluated using Microsoft Excel spreadsheet, by performing a Newtonian algorithm [27] minimizing the sum of squares of errors (SSE) between the experimental data and the model fitted data [24].

The hydrolysis rate cannot be measured directly from the BMP tests experimental data; however, using the first-order hydrolysis model and assuming that the rate limiting step is hydrolysis, valuable elucidations about hydrolysis kinetics can be obtained from the BMP test results [22, 28]. The apparent hydrolysis rate constant k_h (day^{-1}) can be defined from a straight line with a slope, whose magnitude is:

$$-k_h t = \ln[1-(Y/Y_{max})] \quad (2)$$

where Y is the cumulative methane production (mL) at time t (days) obtained from the BMP test. Y_{max} is the ultimate methane yield from BMP assay at the end of the incubation time (mL) that should represent the total concentration of hydrolysable COD at the beginning of the tests. The slope k_h can be obtained by plotting ln $[1 - (Y/Y_{max})]$ versus time and performing linear regression. The modified Gompertz equation was used to fit the BMP assay data from SY1 50 %, SY2 25 %, and SY3 25 %.

Results and discussion

Characterization of SY samples

A summary of SY samples characteristics (SY1, SY2, and SY3) is presented in Table 2. Solids content was mostly VSS which can be related to the yeast cells biomass or microorganisms present in the samples [29]; the TCOD

Table 2 Summary of the SY samples characteristics

Parameter (units)	SY1	V.C.[b]	SY2	V.C.[b]	SY3	V.C.[b]
Beer source IBUs*	48		17.3		55	
Total suspended solids (g/L)	8.2	0.00	11.8	0.02	11.9	0.18
Volatile suspended solids (g/L)	8.2	0.00	10.9	0.03	11.7	0.13
pH	5	0.00	4.2	0.00	5	0
TCOD (g/L)	33.8	0.17	86.4	0.02	92.3	0.03
SCOD (g/L)	21.6	0.00	55.4	0.02	52.4	0.01
Total N (N g/L)	1.5	0.22	3.0	0.01	1.0	0.10
Total P (PO_3–P g/L)	0.1	0.17	0.26	0.07	0.18	0.17
Ammonium (NH_3–N g/L)	0.1	0.25	0.02	0.03	0.03	0.16
Alkalinity ($CaCO_3$ g/L)	1.6	0.10	0.72	0.14	0.79	0.041
Carbohydrates[a] (g/L)	4.0	0.04	21.8	0.11	34.9	0.02
Total proteins (g/L)	7.2	0.09	18.5	0.00	17.1	0.03
VFAs (g/L)	1.4	0.02	2.2	0.01	2.9	0.08
Ethanol (g/L)	6.2	0.04	8.4	0.05	6.7	0.001

* Not measured but reported by sample provider

[a] Based on glucose mass weight (180.16 g/mol)

[b] Calculated variation coefficient

values, in the case of SY1, are moderately low if compared with other complex substrates, such as raw manure (128.9 g/kg of COD), cheese way (128.3 g/kg of COD), or corn leachate (122.3 g/kg of COD) [16]. Based on the value of the soluble COD, more than half of the TCOD is available for biodegradation, being approximately 63, 64, and 57 % of SCOD/TCOD for SY1, SY2, and SY3, respectively, which include the soluble carbohydrates and ethanol measured. The C:N ratio was 22 and 29 for SY1 and SY2, respectively, making them suitable for anaerobic digestion, as they fall in the range of 15–30 [30]; in the case of SY3, a low nitrogen concentration (1 g/L) gives a high C:N ratio (92) which can be addressed by adding nitrogen sources. There is an important difference in the organic matter content: TCODs of SY2 and SY3 are about 2.7 times higher than SY1 TCOD, while SY2 carbohydrates content is 5.5 times higher than SY1, proteins are 2.6 times higher in SY2, and ethanol is 1.3 times higher in SY2; SY3 carbohydrates content is 8.7 times higher than SY1, while proteins are 2.4 times higher confirming heterogeneity of the SY samples. This variability can have important implications in the control and strategy of the anaerobic digestion process for each type of SY treated. The measured pH of the samples was 5.0, 4.2, and 5.0 for SY1, SY2, and SY3, respectively, corresponding to their acidic properties. Since the pH was lower than that needed for the BMP assay (pH 7.0), it had to be adjusted, as mentioned in the methodology [31].

The collection method could also have an effect in the composition variability of the different SY, which were not controlled or monitored in this study. Four Peaks Brewing Co. collects SY from the fermenter by pulling the settled yeast from the tank; other methods used in breweries to collect SY include sedimentation, centrifugation [4], or skimming systems when top-fermenting yeasts are used. Besides, the bitterness variability of the source of the samples, which corresponds to the hops content in beer, could also affect methane production. Hoppy beers with high IBU have shown higher stability under storage conditions, as hops resins have bacteriostatic properties [7, 17] which could affect the bacterial consortium involved in degrading the SY samples that contain hops as particulate matter.

SY1 BMP test and Gompertz-equation fit

The average and the standard deviations of the BMP results for SY1 are presented in Fig. 2 as cumulative CH_4 production as well as the Gompertz equation (1), the model fit well to the empirical data, with $R^2 > 0.99$, as shown in Table 3. In addition, the CH_4 conversion of the initial TCOD for each SY is shown in Fig. 3. At the end of the BMP assay, the TCOD conversion efficiency and methane production were higher for the three dilutions of SY1 than

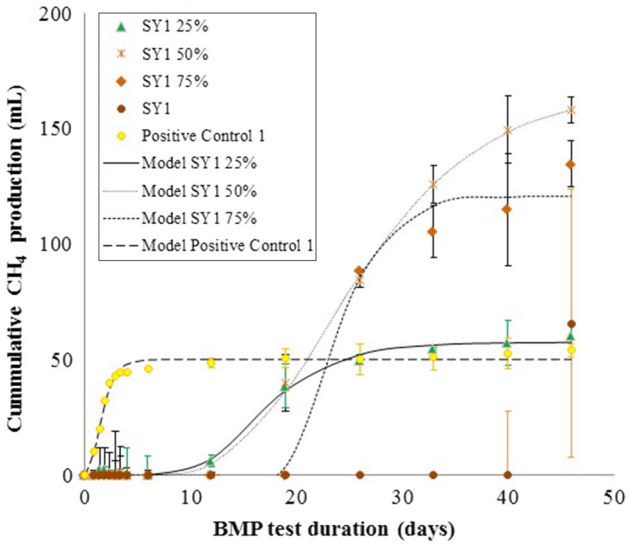

Fig. 2 Cumulative CH_4 production for BMP tests using SY1 at different dilution factors and Gompertz model fit to the empirical data. The average of methane production data is presented here after the subtraction of negative control. The experiments were performed in duplicate, and the standard deviation is represented graphically by *bars*. R^2 was above 0.99 for all the models

for raw SY1. However, the positive control (1) had higher %TCOD removed as methane (58 %) given the availability of acetate. The benefit of dilution is demonstrated for SY1, as 50 and 25 % diluted samples generated the highest methane production at day 45 (74.2 mL CH_4/g COD and 56.6 mL CH_4/g COD, respectively), while for the 75 % diluted sample, methane production was 42.2 mL CH_4/g COD. When treating SY1 without dilution, methane was generated only by the last day of the experiment (66 mL at 45 days or 15.4 mL CH_4/g COD).

Table 3 shows the TCOD balance at the end of the BMP for SY1 substrate. The positive control was calculated as theoretical COD based on acetate concentration, and TCOD equivalent of CH_4 gas was used as 1 mL CH_4 gas = 2.52 mg of COD [28]. At the end of the experiment, the highest TCOD removal as methane (24.2 %) was reached with the 50 % diluted SY1. The SY1 (without dilution) produced the lowest % TCOD removal at the end of the experiment demonstrating a low availability of its TCOD content for methane production. A 58 % acetate TCOD was converted to methane in the positive control one (1).

Gompertz parameters obtained for SY1 long experiment are shown in Table 3 and R^2 is included, which indicates that the model well represented in the experimental data. Specifically, from the model parameters, the rates of methane production seemed to increase with organic load, being lower for SY1 25 % than for SY1 alone, as observed in Fig. 2. However, the lag phases x_o increase with the

Table 3 TCOD balance at the end of the 45 days BMP test for SY1 and Gompertz equation parameters obtained from model fit to the empirical data; correlation coefficient R^2 indicates a good fitting. Gompertz parameters for SY1 (undiluted) were not estimated, since only one measurement point was obtained at the end of the experiments

Sample ID	TCOD to CH_4 (mg)	%TCOD removal as CH_4	P_M (mL)	R_M (mL/day)	x_o (days)	R^2
Positive control 1	136.3	58	50.18	21.60	0.40	0.9917
SY1	165.5	4	–	–	–	–
SY1 75 %	339.4	11	120.80	14.59	19.70	0.9950
SY1 50 %	398.3	19	168.55	7.37	14.12	0.9996
SY1 25 %	151.8	14	57.60	4.63	10.72	0.9982

* Negative control was subtracted from the rest of the experiments

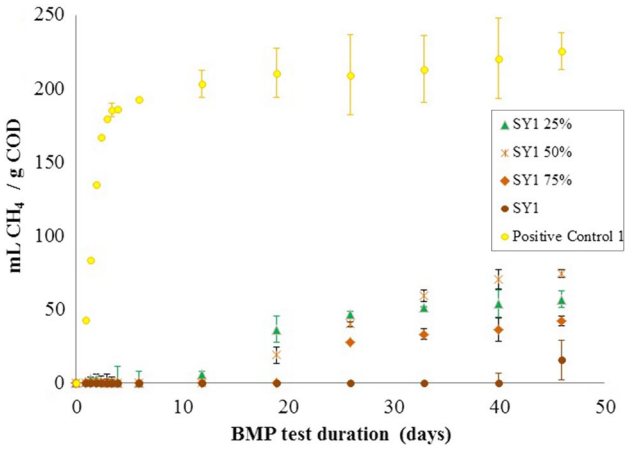

Fig. 3 Ratio of methane production (mL) per initial COD (g) for BMP tests of SY1, SY1 25 %, SY1 50 %, SY1 75 %, and positive control (1). The average of methane production data for all samples is presented here after the subtraction of negative control as well as the standard deviation

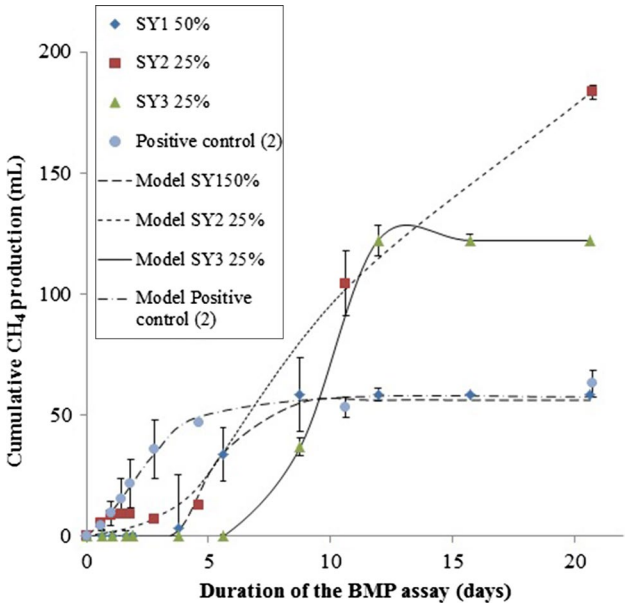

Fig. 4 Cumulative CH_4 production for BMP tests of SY1 50 %, SY2 25 %, and SY3 25 % and the Gompertz equation fit (*line*) to the experimental data. The average of methane production data for all samples is presented here after the subtraction of negative control. The experiments were performed in duplicate, and the standard deviation is represented graphically by *bars*. Estimated R^2 was above 0.99 for all the experiments

organic load. Similar methane potentials were obtained (i.e., 168 mL with SY1 50 %) as that reported for paper sludge (methane potential of 139 mL) by Parameswaran and Rittmann (2012), being a slowly degradable substrate that requires co-digestion to improve methanogenesis [28]. In the case of SY1, the results from the model fit seemed unrealistic since methane production was observed only at the last day of the experiments, thus these results are not presented and longer experiments are suggested.

The different rates at which CH_4 was produced during the BMP assay with SY1 have significant implications on the understanding of the process. The CH_4 production rates obtained in this study (Figs. 2, 3) denote an inhibition on the degradation process of the SY1 sample; the effects of which seem to be alleviated (obtaining faster rates) with a proper dilution. At the end of the 45 day batch experiment with SY1, the biogas started to be produced indicating that this specific SY is slowly-degradable due to its composition or the presence of an inhibitory product non-detected in the previous characterization or due to a gradual acclimatization of the microbial community to the inhibitory conditions. Although a sufficient inoculum biomass concentration was ensured (1.03 inoculum/substrate ratio on gVSS basis) [32], overload might play a significant role on the results obtained when SY1 was fed alone since 67 % of the theoretical minimum inoculum [31] was added. The effect of hops should be explored, since its presence as particulate matter could be affecting the digestion process of the SY1 substrate. Acids from hops, such as humolones and iso-humolones, can be released from the yeast membrane by a change in pH, and these can inhibit bacterial activity [33, 34]. The effect of the possible inhibitory factor is being reduced by the dilutions; however, a lack of acclimation

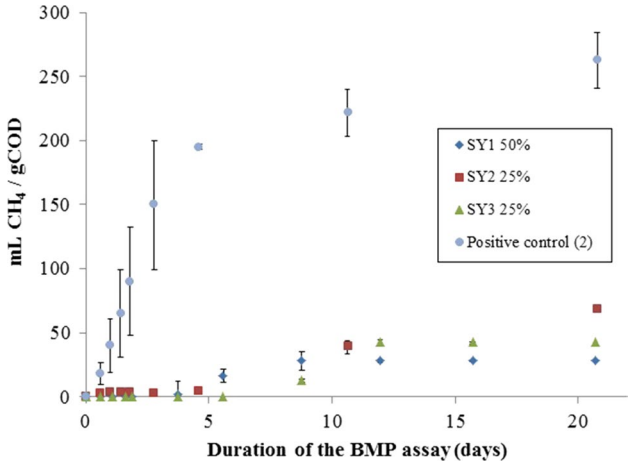

Fig. 5 Ratio of methane production (mL) per initial COD (g) for BMP tests of SY1 50 %, SY2 25 %, and SY3 25 %. The average of methane production data for all samples is presented here after the subtraction of negative control as well as the standard deviation

Table 4 Summary of performance parameters for each tested SY as the observed lag phases and % of the initial TCOD removed as methane

	Initial TCOD (g)	Lag phase (days)	%TCOD removal
SY1 50 %	2.1	3	7
SY2 25 %	2.7	<1	17
SY3 25 %	2.9	6	11
Positive control (2)	0.24	0	67

and origin of the inoculum could also contribute to this effect [35]. A 25 % SY1 achieved its maximum production volume in less time than the rest of the tested dilutions, this probably being related to a less inhibitory effect due to less sample amount; in addition, the smaller degradable fraction was degraded faster. The same effect of substrate concentration can be noticed in the curves of the positive control which only has acetate to be easily degraded. The slow degradability rate of the SY1 and its dilutions (denoted by a lag shape at the beginning of the curve in Figs. 2, 3) can also be related to an insufficient buffering capacity and the acidic properties of the substrate [16]; pH should be monitored [31] in further experiments with SY samples that have similar composition to SY1.

BMP test of different SY

A short BMP test was performed with diluted samples of SY2 and SY3 at 25 % and SY1 at 50 % with the intention of evaluating differences in the methane production performance. Dilutions were chosen based on the initial TCOD values for each SY2 and SY3 to avoid overcharge and were compared to SY1 50 % (around 17 g/L of TCOD), since a better performance was obtained in the previous experiments. In addition, another study observed overload of the anaerobic digestion of SY [15]. Figure 4 shows the results of cumulative methane production from the three samples during 20 days of BMP assay and the model fit (line) to the experimental data, while Fig. 5 shows the conversion of initial g of TCOD of SY to mL of methane.

The highest cumulative methane production was observed at the end of the 20 days assay with SY2 25 %, being 183 mL or 67.9 mL CH_4/g COD. A 122 mL of methane or 42.2 mL of CH_4/g COD was obtained with SY3 25 % and 58 mL or 27.9 mL CH_4/g COD with SY1 50 %, as observed in Figs. 4 and 5. These values were significantly lower than the theoretical methane potentials (mL) presented in Table 5. From the experiment presented before with SY1 and its dilutions and this short experiment, less availability of this sample to be rapidly degraded could be expected compared to SY2 and SY3 substrates. The characteristics of each SY sample related to biodegradability; the experimental conditions and the possible presence of inhibitory products can control the kinetics of the anaerobic digestion which denotes the pattern of the curve of biogas production [16]. Its interpretation can help to prevent digestion issues or select strategies.

The percentage of the initial TCOD that was converted to methane, as %TCOD removal is presented in Table 4. A better performance was reached with SY2 25 %, where 17 % of TCOD was removed as methane in 20 days compared to 7 % and 11 % for SY1 50 % and SY3 25 %, respectively. With higher organic load, SY3 had higher

Table 5 Summary of performance parameters estimated from the Gompertz equation with the SY BMP data

	SY1 50 %	SY2 25 %	SY3 25 %	Positive control 2
P_M (mL)	56.29	200.95	122.23	57.62
R_M (mL/d)	20.83	14.86	150.34	14.29
x_o (day^{-1})	3.90	3.71	8.52	0.34
R^2	0.9961	0.9974	1.0000	0.9927
k_h (day^{-1})	0.0115	0.0123	0.0185	0.1094
Theoretical methane potential* (mL)	833	1071	1151	95

* Based on 1 mL of CH_4 = 2.52 mg of COD [28]

%TCOD removal than SY1 which can also be related to its higher carbohydrate fraction. A highly concentrated SY should also imply a higher concentration of materials that are inhibitory [35]; this implies that the inhibitory effect its present only in certain SY independent from the organic load, as SY1 was fed in low concentration and showed the lowest performance.

Performance model fit to the BMP data

Table 5 presents the parameters obtained the model fitting and their adjustment (as R^2). A good fitting was obtained, thus the Gompertz equation can explain more than 99 % of the variation of the BMP with SY data. From the Gompertz equation parameters (Table 5), we could suggest that SY1 50 % and SY2 25 % are different only in terms of $P_{M,}$ while SY3 25 % presented the longer lag phase (also observed in Table 4) but higher rate of methane production. The P_M was only 6.7, 18.8, and 10.7 % of the theoretical methane potential (Table 5) for SY1 50 %, SY2 25 %, and SY3 25 %, respectively. As mentioned before, similar methane production has been reported [28]. This could be attributed to different factors: slowly degradable substrate, lack of acclimation, origin of the inoculum, and presence of hops in the sample. Moreover, it has been reported that the presence of viable yeast cells decreases methane production [36]. To increase methanogenesis, co-digestion treatment is suggested.

Longer lag phases with SY3 could imply a slow hydrolysis step. From Fig. 4, a better performance was observed with SY2 25 % in 20 days test, and this sample came from the beer source that had the lower content of hops. The acids from hops have been confirmed to inhibit the activity of Gram-positive bacteria [33] which perform the hydrolysis of biopolymers and fermentation of monomers to organic acids, alcohols, hydrogen, and CO_2 [37]. In this context, a slower hydrolysis step, as prolonged lag phases, was noticed with the samples of hoppy beer sources (SY1 and SY3 in Figs. 4 and 5).

Kinetic model fit to the BMP data

Figure 6 shows the plotting of Eq. (2), where the slopes of the linear regression correspond to the hydrolysis constant for each sample exponential phase of methane production. For this analysis, the experimental data that corresponded to the observed or apparent lag phase of each individual experiment were discarded. Table 5 also presents the k_h values and other performance parameters.

From Fig. 6, the average apparent hydrolysis constant for the three SY samples was 0.014 ± 0.003 day^{-1}, while the positive control (2) presented a higher hydrolysis rate

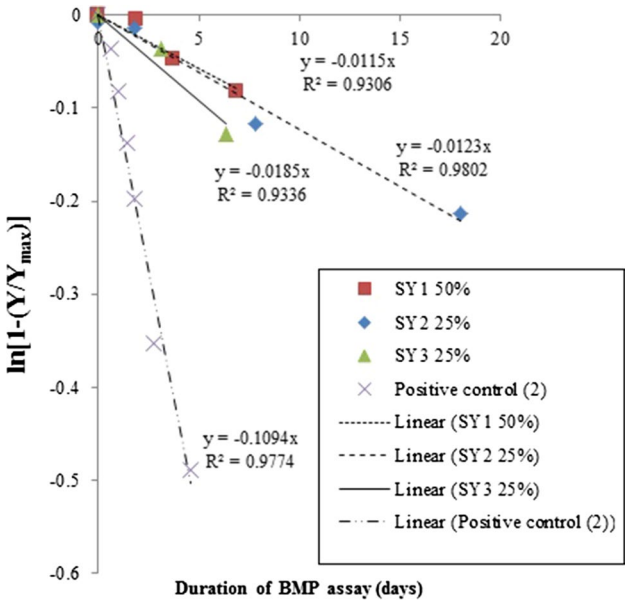

Fig. 6 Linear regression of Eq. (2). Plots from the BMP test data of SY1 50 %, SY2 25 %, and SY3 25 % to estimate the hydrolysis rate constants (k_h). R^2 is also shown in the *graphic*. More than 90 % of the variation was explained and only the exponential phase of methane production was used for the linear regression

(0.1094 day^{-1}) given the simplicity of the fed substrate. From SY results, the highest k_h estimation was obtained with SY3 25 % as 0.0185 day^{-1}, that could be related to a higher easily degradable content. The higher complex composition of SY1 which was slowly degraded is represented by a shorter observed lag phase (Fig. 4; Table 4) and slower hydrolysis rate (0.0115 day^{-1}). Other hydrolysis constants reported in the literature are: 0.22 day^{-1} for waste activated sludge [35] and 0.03 day^{-1} for pig waste [28] which are higher than those obtained for diluted SY indicating its slow degradability. Carbohydrates and proteins have shown faster conversion rates than fats, but lower gas yields [30]; these are part of the readily usable COD fraction of each SY. Since SY3 and SY2 had higher concentrations of these, a higher hydrolysis rate was obtained. The SY2 at 25 % is comparable to the raw SY1 from the previous experiments in terms of carbohydrates concentration, which could explain the difference in bioavailability. SY3 which also has high carbohydrate content might be more complex and substrate inhibition could be occurring initially, thus the longer lag phase is observed denoting a slower hydrolysis step. C:N ratio was high (92) for anaerobic digestion, thus affecting the initial phase, however, the stirring and temperature conditions of the experiment might have induced proteins to break down and ammonia released to the media; this was a problem that affected SY thermophilic anaerobic digestion reported elsewhere [14].

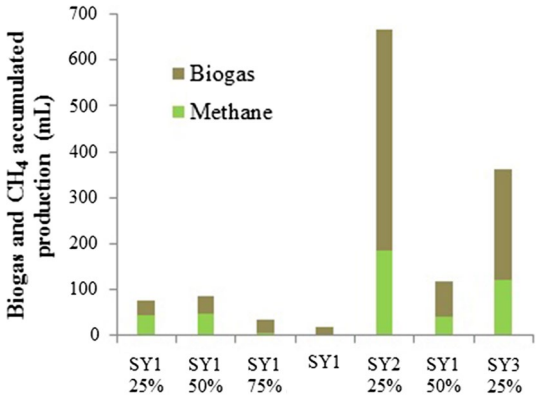

Fig. 7 Biogas and methane accumulated production (mL) at day 20 for all the experiments performed in this study. SY 50 % shows two different results because of the independent experiments performed: the first one that lasted 45 days and the second one that lasted 20 days. Negative controls results were subtracted from the data presented here

Biogas production from SY

Beer sources of the three SY are formulated differently which affects the biogas production rate and the quality [30]. As a mean to comparison, Fig. 7 presents the biogas accumulated production at day 20 for each SY sample treated in this study. Clear differences are observed between each sample, where SY2 25 % had a biogas production of 665 mL and 27 % of this corresponded to methane. On the other hand SY1, 50 % had a biogas production of 80–117 mL composed by 56–35 % methane, respectively. In the case of SY3 25 %, 360 mL of biogas was produced at day 20 with 33 % of methane. In this context, a biogas of good quality and that is appropriate to be used as a fuel has to have a methane composition of 50–80 % [38]. Although SY1 had the lowest volume and rates of methane production, biogas with higher quality was produced, thus for SY2 and SY3, post-treatment considerations are required to increase methane concentration in the whole biogas mixture. Biogas from SY1, that came from a hoppy beer source had higher composition of methane, thus methanogens could have utilized the readily usable substrate from the samples, while fermentation was inhibited generating less of other gases, such as hydrogen and CO_2. The suspected inhibitory effect of hops could explain the biogas composition differences between SY1 50 % and SY2 25 %; however, detailed experiments are suggested.

Acclimation and origin of the inoculum can also be contributing factors, thus the long-term experiments are suggested as well as a better characterization of the inoculum to guarantee certain performance [39]. To achieve higher efficiencies or faster rates in methane production and anaerobic digestion, inhibition factors (such as hops content, buffering capacity, or overcharge) could be addressed through the acclimation of the inoculum or a proper dilution of the SY as well as other authors suggest co-digestion [11, 14, 15]. It should also be considered that different performances can be obtained depending on the formulation of the beer source of the SY to be treated. Using SY as an energy substrate would offer an alternative to inactivation and processing, and an on-site source of energy that can also be beneficial to the brewing industry. In the same way, the inhibitory effect of hoppy SY could be an advantage and be added to control the first stage of anaerobic digestion; however, additional research on this topic is required. From the interpretation of the biodegradability test of SY, important considerations can be taken when advanced treatment technologies, as anaerobic digestion is going to be used.

Conclusions

BMP tests were performed to evaluate SY from different beer sources as potential substrate for methane production. Different dilutions (25, 50, and 75 %) of SY1 were tested, and possible inhibition was noticed as confirmed by Gompertz parameters estimation. The highest TCOD removal (24 % with 150 mL of methane) was obtained with SY1 50 % (45 days). SY2 25 % (20 days) showed 19 % TCOD removal (183 mL of methane, k_h 0.0123 day^{-1}) and shorter lag phase (<1 day) than SY1 and SY3, denoting higher bioavailability. However, a better biogas quality was obtained with SY1 that had slower k_h (0.0115 day^{-1}).

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